Amendments to the Claims

This listing of calims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Amended) A method of preparing a gene vector, said method comprising:

a) transforming yeast cells with a RKO clone and a yeast targeting cassette (YTC), wherein said RKO clone comprises a genomic clone insert, a yeast replication element, a yeast selectable marker, a bacterial origin of replication, optionally a bacterial selectable marker, and optionally a mammalian negative selection marker, and wherein said YTC comprises a bacterial/mammalian positive selection marker flanked by recombinogenic arms, and said YTC does not include a yeast selectable marker;

b) maintaining said yeast cells under conditions wherein said RKO clone and said YTC undergo homologous recombination via said genomic clone insert and said recombinogenic arms to produce a gene targeting vector;

c) selecting transformed yeast cells by their expression of said yeast selectable marker on said gene targeting vector or on said RKO clone;

d) isolating said gene targeting vector and said RKO clone from said selected yeast cells;

e) transforming bacterial cells with said gene targeting vector and said RKO clone;

f) selecting transformed bacterial cells that grow on selective media that is selective for bacterial cells expressing said bacterial/mammalian positive selection marker, thereby selecting for bacterial cells transformed with said gene targeting vector; and

g) isolating said gene targeting vector from said selected bacterial cells.

2. (Original) The method of claim 1 wherein said bacterial cells are Escherichia coli.

3. (Original) The method of claim 1 wherein said RKO clone is a cosmid and further comprises at least 1 Cos site.

4. (Original) The method of claim 1 wherein said RKO clone further comprises a multiple cloning site, and wherein said genomic clone insert is present within said multiple cloning site.

5. (Original) The method of claim 1 wherein said YTC further comprises loxP or FRT sites flanking said mammalian positive selection marker.

6. (Original) The method of claim 1 wherein said RKO clone comprises a mammalian negative selection marker.

7. (Original) The method of claim 1 wherein said YTC is generated by a PCR reaction using chimeric oligonucleotides bearing sequence identity to both the bacterial/mammalian positive selection marker and the GRI.

8. (Original) The method of claim 1 wherein said YTC comprises an internal ribosomal entry site (IRES) element that allows protein translation of said bacterial/mammalian positive selection marker in mammalian cells to occur from mRNA transcripts driven by a promoter in the GRI.

9. (Original) The method of claim 1 wherein said bacterial/mammalian positive selection marker lacks a polyadenylation site on the 3' end thereof.